Total Gas Saturation Considerations for Recirculating Aquatic Systems

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ABSTRACT

Zebrafish (*Danio rerio*) are now widely used in aquatic research facilities for genetic and vertebrate development studies. Most of these facilities utilize recirculating systems for zebrafish production. Dependable production of high-quality fish is of vital concern in these recirculation systems as these fish are valuable and in many cases irreplaceable in terms of their significance to the research being conducted.

Water quality is of utmost concern in zebrafish systems. One critical parameter that has received attention in these facilities is that of total gas pressure. Under abnormal conditions, the partial pressures of dissolved gases in the water can be greater than saturation. When this is the case, there is a potential for problems with gas bubble trauma and an increasing chance for secondary microbial infections. This paper discusses total gas supersaturation theory, problems associated with supersaturation, methods of monitoring total gas pressure, and ways that gas bubble problems can be prevented in recirculating aquatic systems.

INTRODUCTION

Gas Transfer

Under steady-state conditions, the partial pressures of dissolved gases in water are in balance with the pressures of the same gases in the

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atmosphere above the water. Henry's law (Equation 1) is used to determine saturation concentrations of dissolved gases (Colt 1984).

$$C = 1000\ K\ ß\ X\ ((pATM - pH_2O)/760) \quad (1)$$

Where: C = Concentration of the gas (mg/L)

K = Ratio molecular wt of gas to volume (mg/mL)

ß = Bunsen coefficient for the gas

pH_2O = Vapor pressure of water (mm Hg)

pATM = Barometric pressure (mm Hg)

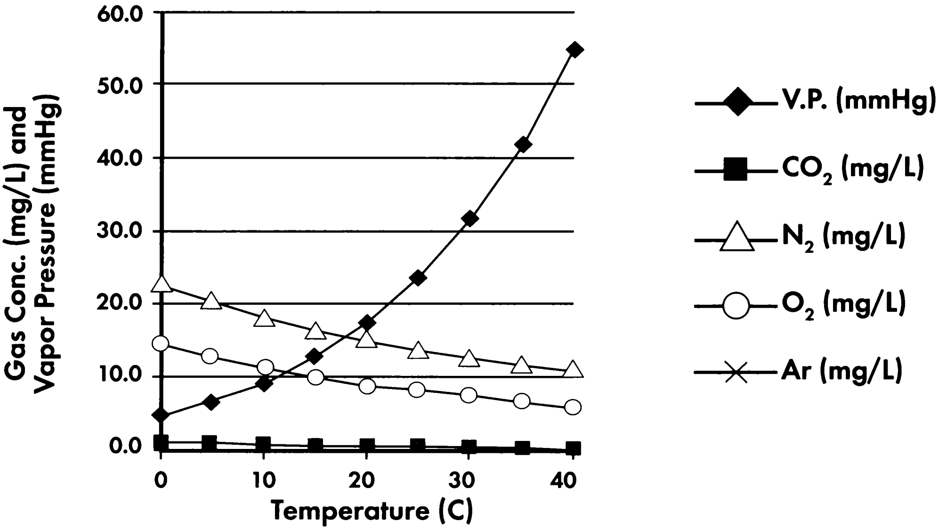
X = Mole fraction of the gas

According to Henry's Law, when the pressure of gas over the water is decreased, the amount of dissolved gas also decreases. In addition, the saturation concentrations of those gases will vary depending on temperature, salinity, and pressure. Higher pressure increases the amount of gas dissolved per unit volume, so the saturation concentration for a gas will be higher in deeper water. The inverse is the case for temperature and salinity. Water at higher temperature or salinity will have less gas per unit volume. Table 1 and Figure 1 present saturation concentrations for various gases in water at different temperatures.

Table 1. Sea level saturation concentrations of dissolved gases and water vapor pressure in freshwater at different temperatures (Colt 1984).

Temp (C)	**N_2 (mg/L)**	**O_2 (mg/L)**	**Ar (mg/L)**	**CO_2 (mg/L)**	**pH_2O (mmHg)**
0	23.0	14.6	0.89	1.09	4.6
5	20.3	12.8	0.78	0.89	6.5
10	18.1	11.3	0.69	0.75	9.2
15	16.4	10.1	0.62	0.63	12.8
20	14.9	9.1	0.56	0.54	17.5
25	13.6	8.2	0.51	0.46	23.7
30	12.6	7.5	0.46	0.40	31.8
35	11.7	6.9	0.42	0.35	42.2
40	10.9	6.4	0.39	0.31	55.3

Figure 1. Sea level saturation concentrations of dissolved gases and water vapor pressure in freshwater at different temperatures (Colt 1984).



The sum of the partial pressures of all dissolved gases plus the vapor pressure of water is referred to as the Total Gas Pressure (TGP). The difference between TGP and atmospheric pressure is defined as Delta P (ΔP). Both TGP and ΔP are usually reported in mm Hg (millimeters of mercury). TGP may also be reported as a percent of sea level or local atmospheric pressure (TGP%). The following equations (Colt 1984) present these relationships:

$$TGP = pN_2 + pO_2 + pCO_2 + pH_2O \quad (2)$$

$$\Delta P = pN_2 + pO_2 + pCO_2 + pH_2O - pATM \quad (3)$$

$$TGP = \Delta P + pATM \quad (4)$$

$$TGP\% = (\Delta P + pATM/\ pATM) \times 100 \quad (5)$$

$$\Delta P = (TGP\% \times pATM)\ 100 - pATM \quad (6)$$

Where: TGP = sum of the partial pressures of all dissolved gases and the vapor pressure of water (mm Hg)

ΔP = difference between TGP and atmospheric pressure (mm Hg)

TGP% = TGP as percent of atmospheric pressure (mm Hg)

pN_2 = partial pressure of dissolved nitrogen (mm Hg)

pO_2 = partial pressure of dissolved oxygen (mm Hg)

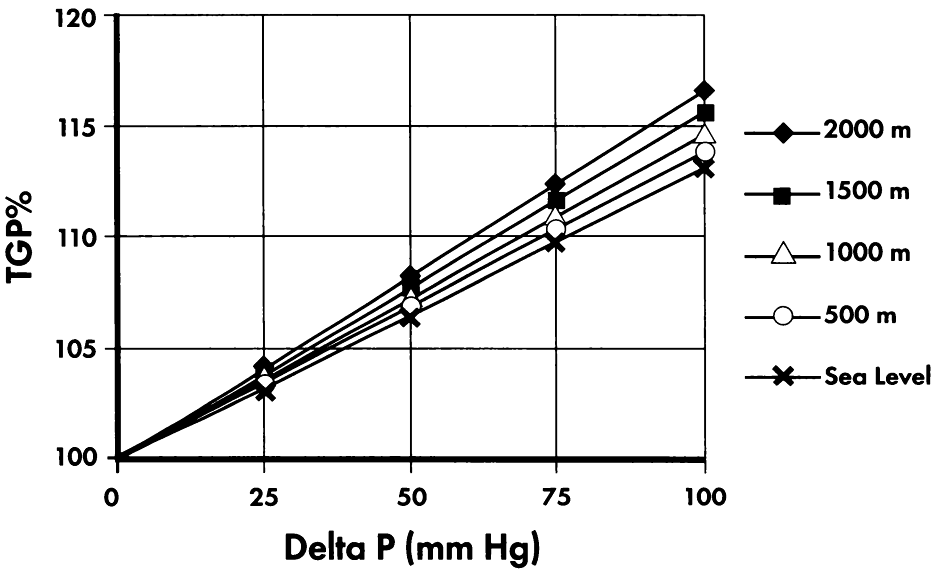
pCO_2 = partial pressure of carbon dioxide (mm Hg)

pH_2O = vapor pressure of water (mm Hg)

pAtm = atmospheric pressure (mm Hg)

Standard Methods for the Examination of Water and Wastewater (APHA/AWWA/WEF 1992) recommends reporting values of ΔP rather than TGP% (percent saturation). However, percent of saturation has been widely used in the past and is probably the most familiar method of reporting total gas pressure data. The problem with the old data is that it was reported in terms of TGP% without the corresponding barometric pressure data. As a result it cannot be accurately converted to ΔP values. Figure 2 presents the relationship between ΔP and TGP% for different elevations.

Figure 2. Relationship between ΔP and TGP% at different elevations.



Measurement of Dissolved Gases

Dissolved gases have been measured using manometry, volumetric tests, mass spectrometry, gas chromatography, chemical titration, direct sensing of pressure, and by headspace partial pressures (Tanner *et al.* 2003; Watten *et al.* 2004). The latter method is the most common means of total dissolved gas measurement and is completed using a saturometer or tensionometer. These devices use a gas permeable membrane (silicone rubber tube) that isolates the dissolved gases and water vapor from the surrounding water. The membrane is connected to a manometer or pressure transducer for pressure measurement.

These instruments measure either ΔP or TGP. When a manometer is used, ΔP (difference between TGP and barometric pressure) is recorded. If a pressure transducer calibrated to absolute pressure is used, then TGP is reported. In this case, ΔP may be calculated using the local barometric pressure. Other instruments utilize a pressure transducer set to zero at the local barometric pressure. The ΔP is reported at that barometric pressure. These devices must be corrected for changes in local barometric pressure.

The actual ΔP experienced by aquatic animals is the difference between TGP and local pressure (barometric pressure plus the hydrostatic pressure). This would be the pressure inside and outside the fish. Gas bubbles will form only when the TGP is greater than the sum of the compensating pressures (APHA/AWWA/WEF 1992; Colt 1984). The compensating pressure is the hydrostatic water pressure, the barometric pressure, and the pressure in the blood or tissues. Gas bubble trauma or gas bubble disease can result if the TDG_{uncomp} is greater than 100% or if the ΔP_{uncomp} is greater than zero (see Equations 7 and 8). The depth of water where the ΔP_{uncomp} is equal to zero is referred to as the hydrostatic compensation depth. Below this depth, it is not possible for dissolved gases to form bubbles or for bubbles to come out of solution. Above this depth, bubbles may form both in the water column and in the blood and tissues of aquatic organisms (Colt 1984).

$$\Delta P_{uncomp} = \Delta P - \rho g Z \qquad (7)$$

$$TGP_{uncomp} = [(pAtm + \Delta P)/(pAtm + \rho g Z)] \times 100 \qquad (8)$$

Where: ΔP_{uncomp} = uncompensated ΔP (mm Hg)

TGP_{uncomp} = uncompensated total gas pressure (mm Hg)

ρ = the density of water (kg/m^3)

g = acceleration of gravity (9.8066 m/s^2)

Z = depth (m)

Before bubble growth can begin, a threshold ΔP must be exceeded. Thus, the ΔP is a direct indicator of the potential for aquatic and marine organisms to develop signs of gas bubble disease.

Causes of Gas Supersaturation

Numerous sources of gas supersaturation have been reported in the literature. Some of these include; 1) spill from dams, 2) power generation cooling water effluent, 3) solar heating, 4) geothermal heating, 5) photosynthesis, 6) groundwater, 7) airlift aeration and gas injection systems, 8) waterfalls, 9) pumping systems, 10) ice formation, 11) barometric pressure changes, 12) aircraft transport, and 13) ocean waves. For additional information on each of these processes, see the publication by Fidler and Miller (1994).

Gas supersaturation seldom develops in recirculating zebrafish growout systems. However, when it has occurred, there have been two primary causes. The first cause is the rapid heating of water that is under pressure. This can occur in systems utilizing temperature-mixing valves. Because gas solubility decreases as the temperature rises, cold supersaturated water can release bubbles as it is warmed (due to the reduced capacity of the warm water to hold dissolved gas). This may be the case when cold tap water (pressurized to about 50 psi) in piping is depressurized to the local atmospheric pressure. A good practice would be to always degas source water prior to use, especially if it has been heated by more than 5° C (10° F).

The other cause of supersaturation in recirculating aquatic systems can be the result of air drawn into a water pump. Within the pump, the air is forced into solution under high pressure resulting in supersaturation. This may be the case if there is an air leak in the piping on the suction side of the pump, if air is introduced in a sump near the pump inlet, or if a vortex forms in the sump or tank near the pump inlet. Air bubbles introduced into the inlet of the pump by one of these methods may result in total dissolved gas supersaturation levels as high as 110% in less than 5 minutes (unpublished data). Thus, it is very important to routinely ensure that none of the following conditions are occurring: air leaks in suction piping, low water levels causing a vortex in sumps, and the introduction of air bubbles near the suction inlet of the pump.

Problems Associated with Gas Supersaturation

Dissolved gas supersaturation has been shown to result in several problems for aquatic animals. Some of these conditions include; 1) bubble formation in the cardiovascular system, 2) over-inflation and/or rupture of the swim bladder, 3) bubble formation in the gills, 4) blistering in the skin particularly around the eyes, 5) bubbles in internal organs, 6) loss of swimming ability, 7) susceptibility to secondary infections, and 8) altered blood chemistry (Weitkamp and Katz 1980, Colt 1986, Fidler and Miller 1994, Speare 1998).

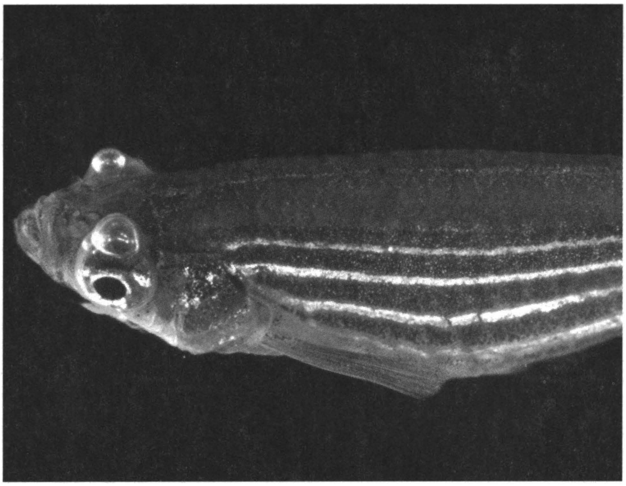


Figure 3. Macroscopic gas bubbles in the tissues around eyes of zebrafish exposed to gas supersaturated water (signs of exophthalmia or "pop-eyed" appearance).

(Photo courtesy of Jennifer L. Matthews, D.V.M., Ph.D., Zebrafish International Resource Center, University of Oregon.)

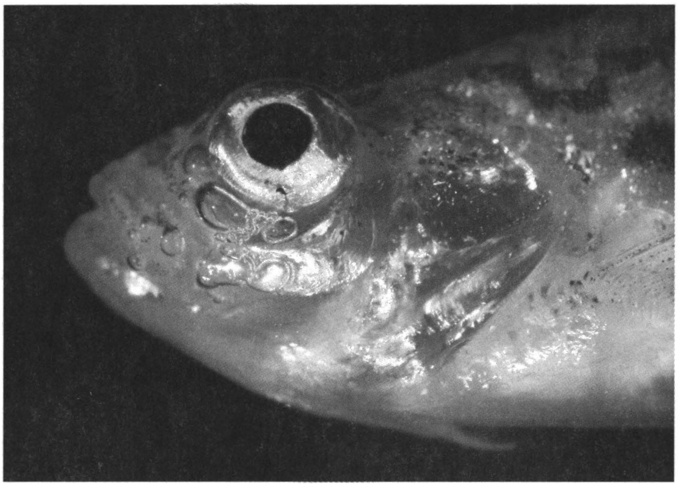


Figure 4. Formation of gas bubbles in tissues near the eyes of zebrafish exposed to gas supersaturated water.

(Photo courtesy of Jennifer L. Matthews, D.V.M., Ph.D., Zebrafish International Resource Center, University of Oregon.)

When zebrafish are exposed to supersaturated water they can show signs of gas bubble disease. This is a non-infectious condition in which gases from supersaturated water come out of solution forming gas bubbles in the circulatory system and tissues of the fish. The major signs of gas bubble disease in zebrafish are exophthalmia (pop-eyed appearance), abdominal distension and hyper-buoyancy, gas bubbles in the skin, or general malaise (Jennifer L. Matthews, DVM, PhD, Zebrafish International Resource Center, University of Oregon, personal communication). The bubbles under the skin can be visible to the naked eye (Figures 3 and 4). This condition can further develop into areas of necrosis, secondary bacterial infections, and eventually death. Diagnosis is based on the observation of gas emboli in capillaries of the gills or internal organs on wet mount exam or by observation of macroscopic gas bubbles in the eyes or skin.

Dissolved Gas Levels of Concern

The U.S. EPA has published a water-quality guideline that recommends a maximum TGP% of 110% of the local atmospheric pressure (U.S. EPA, 1986). This guideline has been accepted by most states. However, there have been numerous studies completed on dissolved gas supersaturation and gas bubble disease since the EPA guideline was developed. These studies suggest that in some cases, the EPA guideline of 110% is too high.

This is definitely the case in shallow applications and/or for certain life stages of aquatic animals. Gas supersaturation values as low as 103% can be dangerous for zebrafish, other aquatic species, or during particular life stages. Adult fish have been shown to be more tolerant of higher total gas pressure than fry or juvenile fishes.

In general, fish will move to deeper water to compress the gases. This prevents bubble formation in their circulatory system and body tissues. However, at a pressure of 103% gas supersaturation, the compensation depth, or depth at which bubbles will not form in the blood of the fish, is only about 12 inches. For every 1% increase in gas tension, the fish must descend 4 inches to compensate for the elevated gas pressure. If the fish tank is only 8-10 inches deep (as is the case with many tanks and aquaria used in research-scale-aquatic systems), the fish would not be able to compensate for a gas supersaturation level of 103%.

CONCLUSIONS

Total dissolved gas pressure is the sum of the partial pressures of all gases dissolved in water plus the water vapor pressure. When the total gas pressure in water is greater than the barometric pressure, the water is supersaturated. These conditions may cause gas bubble disease in fish and other aquatic animals. In lakes and rivers, the aquatic animals can usually move to deeper water to compress the gases. However, for tanks, aquaria, and other shallow settings their vertical movement is limited. Prevention of supersaturation can be extremely important in these cases.

Though instances are rare, it is still possible for supersaturated conditions to occur in laboratory recirculating systems. Supersaturation can result if source water is prepared by mixing cold and warm water, or if air bubbles are drawn into the water pump. Under these conditions, there is an increased chance of gas bubble disease and/or a fish kill. Problems may be minimized or eliminated if source water that has been heated more than 5°C (10° F) is degassed prior to use. In addition, routine care must be taken to ensure that air bubbles do not enter the water pump. This means not allowing the water level of the sump to drop so low that a vortex forms and also ensuring that air bubbles are not drawn into the pump.

REFERENCES

APHA/AWWA/WEF. *Standard Methods for the Examination of Water and Wastewater*, 18th Edition. 1992.

Colt, J. Computation of Dissolved Gas Concentrations in Water as Functions of Temperature, Salinity, and Pressure. *American Fisheries Society Special Publication 14*, 1984.

Colt, J. Gas Supersaturation – *Impact on the Design and Operation of Aquatic Systems. Aquaculture Engineering*, 1986, 5:49-85.

Fidler, L.E., and Miller, S.B. British Columbia Water Quality Guidelines for Dissolved Gas Supersaturation, 1994.

Speare, D.J. Disorders Associated With Exposure to Excess Dissolved Gases. *In Fish Diseases and Disorders*. Wallington, Oxon, UK. 1998. Leatherland, J.F., and Woo, P.T.K., (Eds.). CAB International Publications: Wallington, Oxon, UK.

Tanner, D.Q., Bragg, H.M., and Johnston, H.M. Total Dissolved Gas and Water Temperature in the Lower Columbia River, Oregon and Washington, 2003: Quality-Assurance Data and Comparison to Water-Quality Standards. *Water Resources Investigations Report,* 2003, 03-4306.

U.S. Environmental Protection Agency. *Quality Criteria for Water*. EPA-440-5-86-001. 1986.

Watten, B.J., Boyd, C.E., Schwartz, M.F., Summerfelt, S.T., and Brazil, B.L. Feasibility of Measuring Dissolved Carbon Dioxide Based on Headspace Partial Pressures. *Aquacultural Engineering*, 2004, 30 (3-4):83-101.

Weitkamp, D.E., and Katz, M. A Review of Dissolved Gas Supersaturation Literature. *Transactions of the American Fisheries Society*, 1980, 109:659-702.